



Tropentag 2007  
University of Kassel-Witzenhausen and  
University of Göttingen, October 9-11, 2007

Conference on International Agricultural Research for Development

---

**Preliminary Observations on some Haematological Parameters of Juvenile  
*Heterobranchus Longifilis* Fed Different Dietary Levels of Raw and Boiled Jackbean  
(*Canavalia Ensiformis*) Seed Meal**

**Osuigwe, D., I.,<sup>1</sup> Nwosu C.<sup>2</sup> and Ogunji J. O.<sup>3</sup>**

<sup>1</sup>Dept. of Biotechnology Fed University of Technology Owerri, P.M.B 1526 Owerri, Nigeria

<sup>2</sup>National Veterinary Research Institute, Vom, Nigeria, <sup>3</sup>Dept of Animal Production and Fisheries  
Management, Ebonyi State University PMB Abakaliki, Nigeria.

**ABSTRACT**

Juvenile *H. longifilis* were fed raw and 60 min. boiled jackbean seed meal (JBSM) at different dietary levels for 56 days. Evaluation of some of the haematological parameters showed that haematocrit (PCV), red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) concentration and lymphocytes decreased significantly ( $P < 0.05$ ) with increasing dietary level of JBSM. Boiling JBSM for 60min slightly improved the quality such that fish fed diets with 10% fishmeal substitution had similar PCV and WBC count to those fed the control diet. Other measures to improve the quality of boiled JBSM protein are suggested to enable its use in place of fishmeal at moderate dietary levels in fish production.

**INTRODUCTION**

Given the unavailability and high cost of fishmeal particularly in developing countries, an aggressive search ensued for alternatives to fishmeal that can match its quality as well as be inexpensive. The most viable option appears to be the exploitation of neglected novel legumes, which abound in the tropics (Adeparusi 1994; Osuigwe and Obiekezie, 20007). Jackbean (*Canavalia ensiformis*) is one of such legumes with a crude protein and amino acid profile that recommend it for use as a substitute for fishmeal in fish feed. It is readily available and is hardly consumed by man. It however, has some anti-nutritional factors some of which can be reduced to a very large extent by processing (Udedibie, 1990).

This work is designed to study the effect of feeding raw and processed jackbean seed meal (JBSM) at different dietary levels on some haematological parameters of *H. longifilis* bearing in mind that haematology can be employed to assess fish health (Klinger *et al.*, 1996).

## **MATERIALS AND METHODS**

Two types of JBSM were obtained by milling the raw seed with hammer mill and subjecting a portion of the milled bean to boiling for 60min. Table 1 shows the proximate composition of two types of JBSM used. Thirteen isonitrogenous (CP 30%) and isocaloric (ME 2900 kcal/kg) diets were formulated (Table 2). Diet 1 without JBSM served as control. Diets 2, 3, 4, 5, 6 and 7 had the fishmeal component replaced progressively by raw JBSM at 10%, 20%, 40%, 60%, 80%, and 100% respectively. In diets 8, 9, 10, 11, 12 and 13 60min. boiled JBSM replaced fishmeal at 10%, 20%, 40%, 60%, 80% and 100% respectively. The test diets were assigned randomly to duplicate groups of 20 fish of average total length 18cm in 20 liter plastic aquaria. All fish were fed the prescribed diets twice daily at 3% body weight for 56 days. Water was replaced every 3 days by siphoning. The water quality parameters were monitored daily and mean values were temperature  $28.5 \pm 1^{\circ}\text{C}$ ; pH  $6.8 \pm 0.2$ ; DO  $6.4 \pm 0.5$  mg/l.

Blood samples from the caudal artery using 2ml plastic syringes and needle treated with anticoagulant were collected from 4 fish and put in sample bottles at the commencement of feeding trial and bi-weekly subsequently from each aquarium until the conclusion of the trial. Haematocrit (PCV) was determined with a microhaematocrit centrifuge. RBC and WBC counts were determined with a haemocytometer. Haemoglobin concentration estimates were determined as described by Wedemeyer and Yasutake (1977) while differential leucocyte counts were determined by counting stained (Leishmans') blood smear with a light microscope.

The data were subjected to analysis of variance and the difference between means determined by Duncan's Multiple Range Test.

## **RESULTS AND DISCUSSION**

The PCV, red blood cell and white blood cell counts and haemoglobin concentration decreased significantly ( $P < 0.05$ ) with increasing dietary JBSM (Table 3) such that fish fed the control diet had the highest values that were significantly different from the values obtained from fish fed other diets. Jackbean seed has been shown to contain anti-nutritional factors (Udedibie, 1990; Rajaram and Janardhanam, 1992). Probably the increasing presence of anti-nutritional factors in increasing dietary JBSM caused the inferior haematological parameters observed in fish fed such diets in this work. Concanavalin A found in Jackbean seed agglutinates red blood cells in monogastrics (Liener, 1979;) while saponins are known to cause erythrocyte haemolysis and reduction of blood (Cheeke, 1971). Gossypol found in some legumes was reported to reduce PCV and haemoglobin concentration in rainbow trout (Herman, 1970).

Improvement was observed in the haematological parameters of fish fed boiled JBSM diets relative to those fed raw JBSM diets (Table 4). This result is in agreement with earlier reports that heat treatments reduced the level of anti-nutritional factors in jackbean seed (Udedibie and Carlini 1998). However, the improvement arising from boiling JBSM was limited such that only PCV and WBC count of fish fed at 10% level of fishmeal substitution assumed values similar to those fed the control diet. The limited improvement observed may have resulted from apparent reduction in the quality of boiled

JBSM protein. Bressani *et al.*, (1987) showed that heat treatment not only reduced the level of lysine but also destroyed methionine (both of which are essential amino acids) in jackbean seed thus degrading the biological value of JBSM protein. Moreover essential fatty acid, mineral and vitamin deficiencies or imbalance have been known to reduce red blood cell count, PCV and Hb concentration in fish (Tacon, 1992) and heat treatment, have been known to cause such deficiencies/imbalance in seed legumes (Viola *et al.*, 1983).

The general decrease in circulating lymphocytes observed in this work also agrees with findings of Pickering and Pottinger (1987). In spite of the negative effects arising from increasing dietary level of JBSM, the values of haematological parameters observed in this work were still within the normal range for catfish as reported by Erondy *et al.*, (1993).

## CONCLUSION

It can be concluded that high dietary levels of even boiled JBSM reduced the values of haematological parameters of *H. longifilis* but not below the normal levels. Measures to improve the quality of boiled JBSM protein such as amino acid supplementation should be investigated to enable its use at moderate dietary levels without adverse effects.

## REFERENCES

- Adeparusi, E.O. (1994). Evaluation of the nutritive potential of cooked pigeon pea (*Cajanus cajan*) meal as a plant protein source for *Clarias gariepinus* fingerlings. *J. Agric Tech.* (1): 48-57.
- Bressani, R., Brenes, R.G., Garcia, A and Elias, L.G. (1987). Chemical composition, amino acid content and protein quality of *Canavalia* spp. seeds. *J.Sci. Food Agric.* 40:17-23.
- Cheeke, P.R. (1971). Nutritional and physiological implications of saponins: a review. *Can. J. Anim. Sci.* 51:621-623.
- Erondy, E.S., Nubia, S. and Nwadu, F.O. (1993). Haematological studies on four catfish species raised in fresh water pond in Nigeria *Journal of Applied Ichthyology* 9 (3-4):250-256.
- Herman, R.L. (1979). Effects of gossypol poisoning on rainbow trout (*Salmo gairdneri*). *J. fish Biol.* 2:293-304.
- Klinger, R. C., Blazer, V.S. and Echevarria, C. (1996). Effects of dietary lipid on the haematology of channel catfish *Ictalurus punctatus*. *Aquaculture* 147:225-233.
- Liener, I.E. (1979). Phytohemagglutinins. In: *Herbivores: their Interaction with secondary Plant metabolites* (Rosenthal, G.A. ed), Academic Press, New York, Pp 567-597.
- Osuigwe D.I and Obiekezie A. I. (2007). Assessment of the growth performance and feed utilization of fingerling *Heterobrenchus longifilis* fed raw, and boiled jackbean (*Canavalia ensiformis*) seed meal as fishmeal substitute. *Journal of Fisheries International* 2(I):37-41.
- Pickering, A.D and Pottinger, T.G (1987). Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. *J. Fish Bio.* 30:701-712.
- Rajaram, N. and Janardhanam, K. (1992). Nutritional and chemical evaluation of seeds of *Canavalia gladiata* and *Canavalia ensiformis*, the underutilized food and fodder crops in India. *Plant Foods for Human Nutrition* 42:329-336.

- Tacon, A.G.J. (1992). Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmed fish *FAO Fish Technical Paper* No 330. Rome, FAO, 75p.
- Udedibie, A.B.I. (1990). Nutritional evaluation of Jackbean (*Canavalia ensiformis*) for the Nigerian Poultry Industry, *Ambio* 19(8): 361-365
- Udedibie, A.B.I and Carlini, C.R. (1998). Questions and answers to edibility problem of the *Canavalia ensiformis* seeds-A review. *Animal Feed Science and Technology* 74:95-106.
- Viola, S., Mokady, S. and Arieli, Y. (1983). Effects of soybean processing methods on the growth of carp (*Cyprinus Carpio*). *Aquaculture* 32:27-38.
- Wedemeyer, G.A. and Yasutake, W.T (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. *Technical papers of the US Fish and Wildlife Service* No. 89. Washington, D.C. US Department of the Interior, Fish and Wildlife Service 18p.

Table 1: Chemical composition of Jackbean seed meal (g/kg Dm)

	<b>Raw</b>	<b>Boiled (60 min.)</b>
Crude protein N x 6.25	282.50	254.00
Ether extract	29.00	28.00
Crude fibre	67.30	62.10
Ash	34.40	29.20
NFE <sup>1</sup>	586.80	626.70
Phosphorus	6.20	-
Calcium	0.90	-
Magnesium	0.80	-
Gross energy (Kcal/100g)	459.32	456.52

<sup>1</sup>NFE = Nitrogen Free Extract; <sup>2</sup>Mg = Magnesium; Ca = Calcium

Table 2: Composition of Experimental Diets

	<b>Diet No. /% Fishmeal Substituted with JBSM</b>												
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>
	<b>Control</b>	<b>10%</b>	<b>20%</b>	<b>40%</b>	<b>60%</b>	<b>80%</b>	<b>100%</b>	<b>10%</b>	<b>20%</b>	<b>40%</b>	<b>60%</b>	<b>80%</b>	<b>100%</b>
Fishmeal	22.00	19.80	17.60	13.20	8.80	4.40	0.00	19.80	17.60	13.20	8.80	4.40	0.00
JBSM*	0.00	4.36	8.72	17.44	26.17	38.89	43.61	4.93	9.86	19.71	29.57	39.42	49.28
Maize	35.00	32.84	30.68	26.36	22.03	15.21	12.39	32.27	29.54	24.09	17.63	12.18	6.72
Groundnut meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Wheat bran	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Palm oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Bone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix**	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
% crude	30.27	30.15	29.93	29.50	29.07	28.39	28.11	30.10	29.83	29.28	28.63	28.09	27.54
ME	2986	2975	2964	2943	2920	2903	2933	2973	2916	2937	2967	2943	2918

(Kcal/kg)\*\*\*

\*JBSM: diets 2-7 = raw JBSM; 8-13 = 60min. boiled JBSM : \*\* Vitamin and mineral premix : \*\*\* ME calculated

Table 3: Effect of replacement of fishmeal in diet by JBSM on the haematocrit (PVC) count, White Blood Cell (WBC) count and haemoglobin (Hb) concentration of *H. longifilicis*.

% fishmeal substitution	PCV (%)	Mean values		
		RBC count ( $\times 10^6 \text{ mm}^{-3}$ )	WBCC ( $\times 10^3 \text{ mm}^{-3}$ )	Hb conc (g/100ml)
0	44.14 <sup>a</sup>	2.17 <sup>a</sup>	26.54 <sup>a</sup>	12.18 <sup>a</sup>
10	42.47 <sup>c</sup>	1.96 <sup>b</sup>	24.88 <sup>b</sup>	11.47 <sup>b</sup>
20	41.11 <sup>c</sup>	1.95 <sup>b</sup>	22.89 <sup>c</sup>	10.75 <sup>c</sup>
40	41.44 <sup>c</sup>	1.78 <sup>c</sup>	22.97 <sup>c</sup>	10.37 <sup>c</sup>
60	38.09 <sup>d</sup>	1.67 <sup>d</sup>	21.56 <sup>d</sup>	9.75 <sup>e</sup>
80	37.00 <sup>e</sup>	1.58 <sup>e</sup>	20.05 <sup>e</sup>	9.59 <sup>ef</sup>
100	35.95 <sup>f</sup>	1.44 <sup>f</sup>	19.23 <sup>f</sup>	9.65 <sup>e</sup>

Means on the same column with different superscripts are significantly different (P<0.05)

Table 4: Effect of differently processed JBSM at various fishmeal substitution levels on the haematology of *H. longifilicis*.

Process Type	Fishmeal			Substitution			Level by JBSM			Hb Conc (g/100ml)		
	PCV (%)			RBC ( $\times 10^6 \text{ mm}^{-3}$ )			WBC ( $\times 10^3 \text{ mm}^{-3}$ )			Hb Conc (g/100ml)		
	0	10	20	0	10	20	0	10	20	0	10	20
Control	44.14 <sup>a</sup>	44.14 <sup>a</sup>	44.14 <sup>a</sup>	2.17 <sup>a</sup>	2.17 <sup>a</sup>	2.17 <sup>a</sup>	26.54 <sup>a</sup>	26.54 <sup>a</sup>	26.54 <sup>a</sup>	12.18 <sup>a</sup>	12.18 <sup>a</sup>	12.18 <sup>a</sup>
Raw	44.14 <sup>a</sup>	40.50 <sup>cd</sup>	41.40 <sup>bc</sup>	2.17 <sup>a</sup>	1.82 <sup>c</sup>	1.79 <sup>d</sup>	26.54 <sup>a</sup>	23.94 <sup>b</sup>	20.12 <sup>c</sup>	12.18 <sup>a</sup>	10.78 <sup>c</sup>	9.76 <sup>e</sup>
60min	44.14 <sup>a</sup>	42.16 <sup>ab</sup>	37.79 <sup>e</sup>	2.17 <sup>a</sup>	1.89 <sup>b</sup>	1.87 <sup>b</sup>	26.54 <sup>a</sup>	24.10 <sup>a</sup>	22.02 <sup>b</sup>	12.18 <sup>a</sup>	11.46 <sup>b</sup>	10.30

Means in the same cell with different letters are significantly different (P < 0.05)